9. (Twice Amended) The method of claim 8 wherein the transgenic *E.coli* comprises a phaJ transgene [encodes] encoding a D-specific enoyl-CoA hydratase [gene].

10. (Twice Amended) The method of claim 9 wherein the [hydratase] phaJ transgene [gene] is isolated from a bacterium selected from the group consisting of *R. eutropha, Klebsiella aerogenes, P. putida*, and *Aeromonas caviae*.

15. (amended) The method of claim 11 wherein the organism expresses a polymerase that accepts 3-hydroxyhexanoyl CoA and 3-hydroxybutyryl CoA.

31. (amended) A transgenic bacterium or plant for use in any of the methods of claims [1-27] 1 and 7-30.

Please cancel claims 32 and 33.

Remarks

Claims 2-5, 32 and 33 have been cancelled. Claims 1, 6-10, 15 and 31 have been amended. Support for the amendments to claim 1 can be found, for example, at page 12, lines 18-28; Example 5, wherein PHBH copolymers were formed from butanol in cells expressing PHB polymerase and the thiolase and reductase genes; and further in the Examples. Claim 10 was amended to depend from claim 9. Claim 9 was amended to depend from claim 8 and define the transgene as a *phaJ* structural gene (support can be found, for example, in Example 4).

The present invention is directed to the production of polyhydroxybutyrate-co-polyhydroxyhexanoate. As defined by the amended claims, the method is limited to production of PHAs containing 3HH in *E. coli.*. *E. coli.* do not normally produce PHAs and have not previously been described to produce 3HH copolymers. As defined by the amended claims,

several additional genes can be engineered into the *E. coli.* to increase the amount of substrate available so that production is increased to commercially useful levels. The polymerase gene can also be integrated into the chromosome. Either method: providing pathways to increase the amount of substrate or integrating the polymerase, or both, can be used to increase production of the PHH copolymers. The claimed method relies upon the activities of polymerase/synthase enzymes that accept 3-hydroxyhexanoyl-CoA type molecules and more preferably 3-hydroxybutyryl CoA ***and*** 3-hydroxyhexanoyl-CoA type molecules as substrate.

Rejection Under 35 U.S.C. § 112, first paragraph

Claims 1-27, 31-33 were rejected under 35 U.S.C. § 112, first paragraph, as not being enabled. Applicants respectfully traverse this rejection to the extent that it is applied to the claims as amended.

Solely to facilitate prosecution of this application, the claims have been narrowed to *E. coli*. The cancelled claims to transgenic plants and other organisms will be pursued in a continuation application.

The Examiner asserts that unpredictability of the claimed methods arises from a lack of understanding of both the complex interactions of non-native protein assembly and the perturbation of regulation within the transgenic that may arise when engineered changes to metabolic pathways introduce novel interactions. The Applicants are somewhat confused by the Examiner's assertion of unpredictability in view of the claimed methods allegedly introducing "novel interactions". The Applicants submit that the Examples illustrate successful protein-protein interactions that result in the synthesis of polyhydroxybutyrate-co-

polyhydroxyhexanoate. The characterization of the end-product will dictate whether or not the introduction of a "novel" interaction was successful in the overall process. Methods used to carry out such characterizations are well known in the art, and do not require undue experimentation (see, for example lines 29-10, bridging pages 18 and 19). The Applicants have shown PHBH synthesis *via* transgenic *E.coli* (see Example 2). Plasmid pMBXc12J12 (Example 2) harbors a broad substrate range reductase and a polymerase that accept 3-hydroxyhexanoyl CoA and 3-hydroxybutyryl-CoA. The product polymer composition was based upon the substrate feed glucose and butyrate. The discovery that a broad substrate range reductase and a polymerase that accepts more than one type of substrate could be used in a single pathway dedicated to the synthesis of polyhydroxybutyrate-co-polyhydroxyhexanoate, was a completely unexpected result (resulting in PHBH copolymer containing 1.0% HH co-monomer - See Examples).

It is additionally unclear as to what, or which, interactions the Examiner is asserting are "novel". It must be noted that, while the production of polyhydroxybutyrate-co-polyhydroxyhexanoate in *E. coli via* the claimed enzymes is novel, the interactions between substrate and enzyme are ***not***. As discussed throughout the specification and Examples, each of the claimed enzymes are well characterized and defined by the substrates recognized and the products produced. For example, a phbA thiolase gene encoding an enzyme that converts butyryl-CoA (substrate) and acetyl CoA (substrate) to beta-ketohexanoyl-CoA (product) does not represent a "novel interaction" between substrate and enzyme. The claimed enzymes do *not* physically interact with *one another*. However, substrates and enzymes do interact. The

Examiner has cited "De Luca" (*AgBiotech News and Information*, 1993, Vol. 5, No. 6, pgs. 225N-229N) as confirming a lack of predictability for the claimed methods based upon a poor understanding of *plant* metabolic pathways and their *in vivo* regulation. In view of the newly amended claims, and supportive Examples in the specification, the Applicants submit that transgenic *E. coli* harboring the claimed structural genes for the production of polyhydroxybutyrate-co-polyhydroxyhexanoate is fully enabled.

The Examiner has additionally cited references wherein butyryl Co-A dehydrogenase (BCD) activity was not detected in transformed *E. coli* ***extracts*** as support for the assertion that the claims lack enabling support. One of ordinary skill in the art would readily appreciate that an *E. coli* extract, used as a system to provide the necessary cellular machinery for proper expression and post-transcriptional and translational modifications that may be required for any enzymatic activity, is not the same as using an intact *E. coli* transgenic cell for the production of polyhydroxybutyrate-co-polyhydroxyhexanoate, as claimed. The applicants submit that the cited references under 35 U.S.C. § 112, first paragraph, discuss the characterization of secondary metabolic pathways, and butyryl Co-A dehydrogenase (BCD) activity, in "systems" (i.e. plants and cell extracts) that are completely unrelated to an intact *E. coli* cell system. Levels of predictability obviously vary across different systems with regard to the amount of experimentation required to produce the desired outcome. The cited references do not apply to the state of the art (genetics of an intact bacterial system) and therefore should not be further considered as factors to be considered for a determination of the state of the prior art.

Claims 1-27, 31-33 were rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventor had possession of the claimed invention. Applicants respectfully traverse this rejection to the extent that it is applied to the claims as amended.

The Examiner continues to assert that there is no description of the composition or structure for any of the genes set forth in the claims. The Examiner further asserts that the specification teaches only gene names describing genetic loci or abbreviated assessments of phenotypic effects. The Examiner is, again, respectfully reminded that omission of what is generally known in the art does not render the description insufficient. The specification is more than adequate in view of the claimed transgenic *E.coli* harboring *known structural* genes and their encoded enzymatic activities. Indeed, the very knowledge that the genes are known and have been described in the prior art is acknowledged in the background of the invention.

The Examples clearly show possession of the claimed invention. Example 1 provides a step-by-step procedure for isolating the PHB polymerase gene from bacterial cells and incorporating the desired modifications for integration. Analogous procedures are commonly used in the art for the isolation and *in situ* or transgenic expression of many genes. As stated at lines 11-15 of page 21, genes may be isolated and expressed in *E.coli* using the procedure exemplified in Example 1. The applicant respectfully reminds the Examiner that the inquiry into adequate written description is not performed in a vacuum. "Knowledge of one skilled in the art is relevant to meeting [the written description] requirement." *Enzo Biochem, Inc. v. Gen-Probe,*

Docket No. 01-1230 (Fed. Cir. Apr. 2, 2002) (slip op.). This fact has implications not only for validity challenges, but also for patent prosecution. *See In re Alton*, 76 F.3d 1168, 1174-75 (Fed. Cir. 1996).

The claimed methods are further supported in Example 2, which provides an *in vivo* route for the synthesis of PHBH from butyrate using a broad substrate range reductase and polymerase that accepts 3-hydroxyhexanoyl CoA. Example 3 provides for the synthesis of PHBH using the enzymes of the butyrate fermentation pathway. The genes and their sources are disclosed along with step-by-step procedures for gene isolation and cloning. Example 4 provides for PHBH synthesis in *E. coli* using a fatty acid oxidation pathway (*a completely novel method of production*). The gene encoding enoyl-CoA hydratase was isolated from *A. caviae* using the disclosed primers in conjunction with PCR. The enoyl-CoA hydratase enzyme completed the pathway necessary for the *E.coli in vivo* production of PHBH co-polymer. Example 5 teaches the production of PHBH copolymers from butanol in *E.coli* expressing the PHB polymerase, thiolase, and reductase genes. The genetically engineered cells produced a PHBH copolymer containing 1.2% HH. Example 6 teaches PHBH synthesis in *E.coli* harboring a constitutive fatty acid biosynthetic regulon. The methods used to isolate genes encoding the enzymes that facilitate the essential activity of converting ACP to CoA transacylation are also described in detail in Example 6.

The Examiner has referred to case law wherein "the use of generic statements such as vertibrate insulin cDNA or mammalian insulin cDNA without more, is not an adequate written description of the genus because it does not distinguish the genus from the others". *University of*

California v. Eli Lilly and Co., 119F.3d 1559, 1568, 43 USPQ2d 1398, 1406 (Fed. Cir. 1997). However, as shown above, and previously submitted in the response to the Office Action mailed on March 26, 2001, each of the genes harbored by the claimed transgenic organism have defined structural and functional characteristics (for example, their encoded products recognize *known* substrates and produce known products). The claimed genes have been isolated and used as transgenes to produce the desired PHBH's, *as shown in the Examples.*

More relevantly, applicants are not claiming the isolated structural genes, but rather the organisms containing the known or readily obtainable genes. There has been **no** Court decision stating that it is necessary to provide the gene sequences when the genes are known and publicly available, and indeed the recent decisions by the Court of Appeals for the Federal Circuit have confirmed that one need not disclose all that is known to comply with the written description requirement. As the Patent Office frequently quotes in office actions, relying on University of California v. Eli Lilly, the written description requirement is intended to show that applicants have the invention. That is amply demonstrated in this case by the examples showing full reduction to practice prior to filing of the application.

Rejection Under 35 U.S.C. § 103

Claims 1, 6, 8, 9, 15, and 31 were rejected under 35 U.S.C. § 103(a) as being unpatentable over *J. of Bacteriol.*, Vol. 179:4821-4830, 1997 by Fukui *et al.* ("Fukui"), in view of U.S. Patent No. 5,238,833 to Sanders *et al.* ("Sanders"), U.S. Patent No. 5,470,727 to Mascarenhas *et al.* ("Mascarenhas"), and U.S. Patent No. 6,316,262 to Huisman *et al.*

("Huisman"). Applicants respectfully traverse this rejection to the extent that it is applied to the claims as amended.

The discovery that high levels of PHH copolymers could be produced in E. coli is unexpected based on the prior art, which had not been able to demonstrate polymer production before. Applicants have relied on two discoveries: selection of the polymerase (optionally finding much better results through integration of the gene encoding the polymerase) and introduction of enzymes that increase the available substrate for the polymerase. Applications have further utilized a broad substrate range reductase and a polymerase that accepts more than one type of substrate in a single pathway dedicated to the synthesis of polyhydroxybutyrate-co-polyhydroxyhexanoate, resulting in PHBH copolymer containing 1.0% HH co-monomer in high quantities. The prior art (in particular, Fukui) is directed to polymer production in *A. eutrophus*. Sanders, Mascarenhas, and Huisman make use of *separate* pathways (i.e. separate synthases/polymerases) to generate their polymers.

Fukui

Fukui teaches the complementation of PHA negative mutants of *A. eutrophus* and *P. putida* (see table 2, and page 4823). Fukui assays enoyl-CoA hydratase activity in soluble *E. coli* extracts. Fukui does not teach the production of polyhydroxybutyrate-co-polyhydroxyhexanoate in whole cell *E. coli*.

Sanders

Sanders teaches the production of endogenous and exogenous polypeptides with the "transformed hosts harboring the introduced vehicle in a stable way by integration of the vehicle..". Sanders teaches the introduction of extrachromosomal DNA into *Bacilli* hosts, "including the genes for which enhanced expression in the *Bacillus* host is desired" (see lines 66-3, bridging columns 3 and 4). Sanders does not teach the production of polyhydroxybutyrate-co-polyhydroxyhexanoate in *E. coli*.

Mascarenhas

Mascarenhas teaches the insertion of heterologous genes into the chromosome of a host cell such as *E. coli*. Mascarenhas teaches the expression of an integration enzyme, such as an integrase, that causes the integration of the chromosomal transfer DNA into the host cell chromosome. Mascarenhas does not teach the production of polyhydroxybutyrate-co-polyhydroxyhexanoate in *E. coli*.

Huisman

Huisman teaches the integration of a gene encoding a 4-hydroxybutyryl-CoA transferase into bacteria. Huisman does not teach the production of polyhydroxybutyrate-co-polyhydroxyhexanoate in *E. coli*.

Summary

None of the above cited references teach the *in vivo* production of polyhydroxybutyrate-co-polyhydroxyhexanoate in *E. coli* cells. As discussed above, Fukui assays enoyl-CoA hydratase activity in soluble *E. coli* extracts and fails to comment further with regard to this

finding and/or relating it to the production of polyhydroxybutyrate-co-polyhydroxyhexanoate in *E. coli* cells. One of ordinary skill in the art would not have a reasonable expectation of success of the claimed method in view of any combination of the above-identified references. In any case, the Applicants have obtained unexpected results to rebut the *prima facie* obviousness rejection, to the extent one has been made.

Claims 11-14 were rejected under 35 U.S.C. § 103(a) as being unpatentable over *J. of Bacteriol.*, Vol. 178:3015-3024, 1996 by Boynton *et al.* ("Boynton"), in view of U.S. Patent No. 5,238,833 to Sanders *et al.* ("Sanders"), U.S. Patent No. 5,470,727 to Mascarenhas *et al.* ("Mascarenhas"), and U.S. Patent No. 6,316,262 to Huisman *et al.* ("Huisman"). Applicants respectfully traverse this rejection to the extent that it is applied to the claims as amended.

Claim 11 depends from claim 8, which depends from claim 1. Claim 11 incorporates the limitations of claim 1 wherein the production of polyhydroxybutyrate-co-polyhydroxyhexanoate is produced by *E. coli*.

Boynton

Boynton teaches enzyme activity assays showing that crotonase and β-hydroxybutyryl-coenzyme A (CoA) dehydrogenase (BHBD) are highly overproduced in cell *extracts* from *E. coli* harboring the butyryl-CoA synthesis operon. Boynton fails to teach the production of polyhydroxybutyrate-co-polyhydroxyhexanoate in *E. coli* cells.

Sanders

Sanders teaches the production of endogenous and exogenous polypeptides with the "transformed hosts harboring the introduced vehicle in a stable way by integration of the

vehicle..". Sanders does not teach the production of polyhydroxybutyrate-co-polyhydroxyhexanoate in *E. coli*.

Mascarenhas

Mascarenhas teaches the insertion of heterologous genes into the chromosome of a host cell such as *E. coli*. Mascarenhas does not teach the production of polyhydroxybutyrate-co-polyhydroxyhexanoate in *E. coli*.

Huisman

Huisman teaches the integration of a gene encoding a 4-hydroxybutyryl-CoA transferase into bacteria. Huisman does not teach the production of polyhydroxybutyrate-co-polyhydroxyhexanoate in *E. coli*.

Summary

As stated in the foregoing discussion, none of the cited references, singly or in any combination, teach the production of polyhydroxyhexanoate copolymers in *E. coli*. None teach that one will obtain high yields by incorporating enzymes to increase the amount of available substrate. None teach that integration of a polymerase will increase yield in *E. coli*.

In summary, the prior art fails to teach the critical combinations of the elements that yield very high yields. The prior art also fails to lead one skilled in the art to select such elements, evenly individually, and the motivation to lead those skilled in the art to combine them as applicants have done, with a reasonable expectation of success. Indeed, the prior art leads one away from what applicants have done, teaching that one must use other organisms to make PHH copolymers, and that in no case does one obtain high yield.

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MARKED UP VERSION OF AMENDMENTS PURSUANT TO 37 C.F.R. § 1.121

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Marked Up Version of Amended Claims
Pursuant to 37 C.F.R. § 1.121(c)(1)(ii)

1. (Three times amended) A method for the biological production of polyhydroxyalkanoate containing 3-hydroxyhexanoate comprising growing a transgenic [organism selected from the group consisting of a transgenic bacterium and a transgenic plant] *E. coli* having at least one bacterial transgene encoding [an enzyme selected from the group consisting of] a PHA polymerase incorporating C_6 substrates [integrated into the genome] and at least one [a D-specific enoyl-CoA hydratase] enzyme selected from the group consisting of a phbA thiolase gene encoding an enzyme that converts butyryl-CoA and acetyl CoA to beta-ketohexanoyl-CoA, a phbB reductase gene that encodes an enzyme that converts beta-ketohexanoyl-CoA to beta-hydroxyhexanoyl-CoA, a phbC polymerase gene that encodes an enzyme that polymerizes 3-hydroxybutyryl CoA, a D-specific enoyl-CoA hydratase and ß-hydroxyacyl-ACP-coenzymeA transferase, and providing feedstocks for the transgenic *E. coli* [integrated into the chromosome], wherein the production of polyhydroxybutyrate-co-[polyhydroxyvalerate] polyhydroxyhexanoate [containing 3-hydroxyhexanoate] by the transgenic [organism] *E. coli* occurs.

Please cancel claims 2-5.

6. (Twice Amended) The method of claim 1 wherein the phbC polymerase gene [encodes] encoding a PHA polymerase enzyme that incorporates C_6 substrates is incorporated into the bacterial chromosome.

7. (amended) The method of claim [6] 1 wherein the polymerase is from *Aeromonas caviae, Comamonas testosteroni, Thiocapsia pfenigii, Chromatium vinosum, Bacillus cereus, Nocardia carolina, Nocardia salmonicolor, Rhodococcus ruber, Rhodcoccus rhodocrous,* and *Rhodospirilum rubrum.*

8. (amended) The method of claim 1 wherein the [organism] transgenic *E.coli* directs metabolites to production of 3-hydroxyhexanoyl-CoA.

9. (Twice Amended) The method of claim 8 wherein the transgenic *E.coli* comprises a phaJ transgene [encodes] encoding a D-specific enoyl-CoA hydratase [gene].

10. (Twice Amended) The method of claim 9 wherein the [hydratase] phaJ transgene [gene] is isolated from a bacterium selected from the group consisting of *R. eutropha, Klebsiella aerogenes, P. putida,* and *Aeromonas caviae.*

11. The method of claim 8 wherein the organism has the genes encoding the enzymes in a butyrate fermentation pathway.

12. The method of claim 11 wherein the enzymes in the butyrate fermentation pathway are from *Clostridium acetobutylicium* or *Thermoanaerobacterium thermosaccharolyticum.*

13. The method of claim 11 wherein the organism converts butyrate to butyryl CoA or butyryl CoA to crotonyl CoA.

14. The method of claim 11 wherein the organism expresses a broad range reductase that is active on C_6 substrates.

15. (amended) The method of claim 11 wherein the organism expresses a polymerase that accepts 3-hydroxyhexanoyl CoA and 3-hydroxybutyryl CoA.

16. The method of claim 11 wherein the organism expresses a thiolase accepting acetoacetyl CoA.

17. The method of claim 11 wherein the organism expresses an enzyme selected from the group consisting of thiolases specific for 3-ketohexanoyl CoA, reductase active on 3-ketohexanoyl CoA, and 3-hydroxyhexanoyl CoA.

18. The method of claim 8 wherein the organism expresses one or more fatty acid biosynthetic enzymes.

19. The method of claim 18 wherein the fatty acid biosynthetic enzymes are enzymes converting acyl ACP to acyl CoA.

20. The method of claim 19 where the enzymes are selected from the group consisting of ACP-CoA transacylase, acyl ACP thioesterase, and acyl CoA synthase.

21. The method of claim 20 wherein the enzymes are acyl ACP thioesterase and acyl CoA synthase.

22. The method of claim 18 wherein the enzymes are from *E. coli*.

23. The method of claim 8 wherein the organism expresses one or more enzymes forming a fatty acid oxidation complex.

24. The method of claim 23 wherein the one or more enzymes are selected from the group consisting of enzymes epimerizing S-3 hydroxyhexanoyl CoA and enzymes reducing 3-ketohexanoyl CoA.

25. The method of claim 24 wherein the enzymes are from *Nocardia salmonicolor*.

26. The method of claim 24 wherein the enzymes epimerizing S-3 hydroxyhexanoyl CoA are from the *Pseudomonas putida* FaoAB complex.

27. The method of claim 23 wherein the organism accumulates 3-ketohexanoyl CoA due to a lack of a thiolase.

31. (amended) A transgenic bacterium or plant for use in any of the methods of claims [1-27] 1 and 7-30.

Please cancel claims 32 and 33.

Allowance of claims 1 and 6-31 is respectfully solicited.

Respectfully submitted,

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Date: July 29, 2002

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I hereby certify that this paper, along with any paper referred to as being attached or enclosed, is being deposited with the United States Postal Service on the date shown below with sufficient postage as first-class mail in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

Aisha Wyatt

Date: July 29, 2002